Factors affecting allergen-specific IgE serum levels in cats

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Abstract

Pruritic skin diseases are common in cats and demand rigorous diagnostic workup for finding an underlying etiology. Measurement of a serum allergen-specific IgE in a pruritic cat is often used to make or confirm the diagnosis of a skin hypersensitivity disease, although current evidence suggests that elevated allergen-specific IgE do not always correlate with a clinical disease and vice versa. The aim of the study was to to assess the possible influence of age, deworming status, lifestyle, flea treatment, and gender on allergen-specific IgE levels and to evaluate the reliability of IgE testing in predicting the final diagnosis of a pruritic cat. For this purpose sera of 179 cats with pruritus of different causes and 20 healthy cats were evaluated for allergen-specific IgE against environmental, food and flea allergens using the Fc-epsilon receptor based enzyme-linked immunosorbent assay (ELISA) test. The results of the study showed positive correlation between age, outdoor life style, absence of deworming, absence of flea control measures and levels of allergen-specific IgE. Gender and living area (urban versus rural) did not seem to affect the formation of allergen-specific IgE. According to these findings, evaluating allergen-specific IgE levels, is not a reliable test to diagnose hypersensitivity to food or environmental allergens in cats. On the contrary, this test can be successfully used for diagnosing feline flea bite hypersensitivity.

Résumé

Les maladies cutanées pruritiques sont fréquentes chez les chats et exigent un travail rigoureux de diagnostic pour trouver une étiologie sous-jacente. La quantification des IgE sériques spécifiques d'allergène chez un chat pruritique est souvent utilisée pour poser ou confirmer le diagnostic d'une condition d'hypersensibilité cutanée, malgré que les données courantes suggèrent qu'une augmentation des IgE spécifiques d'allergène n'est pas toujours corrélée avec une maladie clinique et vice versa. L'objectif de l'étude était d'évaluer l'influence possible de l'âge, du statut de vermifugation, du style de vie, du traitement pour les puces, et du genre sur les niveaux d'IgE spécifiques d'allergène et d'évaluer la fiabilité d'éprouver les IgE à prédire le diagnostic final chez un chat pruritique. À cette fin, le sérum de 179 chats avec du prurit associé à des causes différentes et 20 chats en santé ont été évalués pour les IgE spécifiques d'allergène contre des allergènes d'origine environnementale, alimentaire et de puces au moyen d'une épreuve immuno-enzymatique (ELISA) basée sur l'utilisation du récepteur Fc-epsilon. Les résultats de l'étude ont montré une corrélation positive entre l'âge, un style de vie à l'extérieur, l'absence de vermifugation, l'absence de mesure de limitation des puces et les niveaux d'IgE spécifiques d'allergène. Le genre et l'environnement de vie (urbain versus rural) n'ont pas semblé affecter la formation d'IgE spécifiques d'allergène. Selon ces résultats, l'évaluation des niveaux d'IgE spécifiques d'allergène n'est pas une épreuve fiable pour diagnostiquer une hypersensibilité aux allergènes alimentaires ou environnementaux chez les chats. Par contre, cette épreuve peut être utilisée avec succès pour diagnostiquer l'hyprersensibilité féline aux piqûres de puces.

(Traduit par Docteur Serge Messier)

Introduction

Hypersensitivity dermatitides are frequent causes of pruritus in cats. Affected animals usually present with one of the following reaction patterns: head and neck excoriations, self-induced symmetrical alopecia, miliary dermatitis, or eosinophilic dermatitis (mainly eosinophilic plaques or granulomas). Food, flea, and environmental allergens are postulated to be the main offending factors.

A role for allergen-specific IgE in these conditions is likely but has not been extensively studied (1–4). Allergen-specific IgE has been detected in sera of allergic but also in healthy and specific pathogen free (SPF) cats (5,6). Allergic cats often present with positive skin tests but one cannot exclude that such reactions, in some instances, may be associated with non IgE-mediated mechanisms and are not uncommon in healthy cats (7). Indeed, non IgE-mediated reactions may play a role in the development of hypersensitivities in cats (8).

In fact, the causal association between allergen-specific IgE and hypersensitivity dermatitides in cats is mainly supported by some studies demonstrating clinical improvement after allergen-specific immunotherapy based on intradermal or allergen-specific IgE tests (2,9,10). It is, however, noteworthy to mention that favorable response to such therapy does not necessarily imply that the underlying mechanisms are IgE-mediated.

Using feline allergen-specific IgE tests in the clinical context is also problematic. One experimental study on feline asthma recently showed that a laboratory, using enzymoimmunometric assay for

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detection of allergen-specifc IgE, had completely unreliable results. The same study demonstrated that intradermal testing had a higher sensitivity while one FcεR1α based ELISA test had a better specificity (11).

These data collectively suggest that high allergen-specific IgE serum levels may not only mirror allergic sensitization but, on the contrary, may also be provoked by other factors and that allergen-specific IgE may not be the only offending factors leading to hypersensitivity reactions.

Alternatively, one can hypothesize that some factors may lead to asymptomatic allergic sensitization and that a high level of allergen-specific IgE may be necessary but not sufficient to induce clinical disease.

Current knowledge on feline IgE and correlation with clinical presentations, especially parasitic diseases and skin, respiratory, and gastrointestinal hypersensitivity reactions have been extensively reviewed recently (12).

The goal of the study was to characterize the allergen-specific profiles of numerous cats. The tested hypotheses were that several factors such as deworming, indoor/outdoor living, flea treatment or gender may influence the development of such IgE and that IgE test is not adequate to make a diagnosis of hypersensitivity dermatitis in cats. The latter information can be regarded as especially important because allergy tests are designed to select allergens for allergen-specific immunotherapy but are sometimes inappropriately used to make or confirm the diagnosis of an allergic disease.

Sera from healthy and pruritic cats were consequently evaluated using a FcεR1α based ELISA test. Pruritic cats were affected by hypersensitivity or non hypersensitivity related diseases. All sera were tested for several environmental, food, and flea allergens and results were statistically analyzed.

Materials and methods

Animals and experimental design

Pruritic cats

Sera of 211 pruritic cats were collected. All pruritic pet cats were all examined by a group of experienced dermatologists (the authors) in Estonia, Belgium, Germany, Sweden, and Switzerland in the context of their practices.

Cats were included provided that they presented with chronic (> 2 episodes or more than two-month duration) pruritus and that a definitive diagnosis had been made.

At the time of inclusion, investigators collected history and clinical information and recorded several parameters including the age, gender, breed, way of life, food, lesions and their localizations, presence of concomitant clinical signs, outcomes of previous treatments, results of performed tests, including blood panels, also treatment outcome and diagnosis. The minimal work-up needed for all cases was adequate flea control, skin scrapings and a fungal culture. Cytological examinations were carried out when necessary, namely when the skin presented with signs of inflammation.

Diagnoses of non-hypersensitivity conditions (other diseases: OD) had to be based on at least one positive result of one specific test and a positive response to an adequate treatment (such as, positive fungal culture and response to antifungal treatment, positive skin scraping, and response to acaricide treatment).

The diagnosis of Non-Flea hypersensitivity dermatitis (Non-Flea HD) was based on the exclusion of all other resembling conditions, the presence of compatible clinical signs (head and neck excoriations, self-induced symmetrical alopecia, miliary dermatitis or eosinophilic dermatitis), and a positive response to either glucocorticoids, ciclosporine or type I antihistamines.

A six- to eight-week restriction diet followed by a two-week challenge with the previous diet (a dietary restriction-provocation test) was carried out whenever possible but it was not a prerequisite for inclusion. Cats with signs with Non-Flea HD responding completely to this procedure were subsequently recorded as having food-induced hypersensitivity dermatitis (Food HD).

Cats with Non-Flea HD not responding to this procedure were included in the group Non-Flea/Non-Food HD and were consequently suspected to develop hypersensitivity reactions in association with environmental allergens.

Cats not subjected to a dietary restriction-provocation test or with inconclusive responses, especially cats improving during the trial but not relapsing during the challenge and outdoor cats not improving during the trial (because Food HD cannot be excluded in these individuals) were included in the undetermined hypersensitivity dermatitis (UHD) group.

The diagnosis of flea bite hypersensitivity dermatitis (Flea HD) was made in pruritic cats with compatible clinical signs, which responded completely to an adequate flea treatment.

Healthy cats

Twenty privately owned physically healthy cats without any present or prior history of allergy signs (namely pruritus), chronic diarrhea, or respiratory signs, were selected as the control population. Non-allergic pruritic cats (OD group) and healthy cats were finally merged in a non-allergic cats group.

Serum samples

Blood was drawn by puncture of the cephalic or jugular vein, allowed to clot at room temperature, and centrifuged. The sera were stored at −20°C until being sent to Heska AG laboratory, Fribourg, Switzerland, for allergen-specific IgE measurements. This laboratory was chosen because the test used had a high specificity in another study (11). This part of the study was approved by the appropriate local authorities.

Measurement of allergen-specific IgE

Collected sera were evaluated in a blinded fashion by Heska AG (Fribourg, Switzerland). Measurement of allergen-specific IgE was done using the Fc-epsilon receptor-based enzyme-linked immunosorbent assay (ELISA) described elsewhere (13,14). The reliability of this test has been demonstrated previously in several studies (11,13,14).

All sera were tested against a panel of 48 allergens including flea saliva, environemental allergens, and food allergens (see Appendix I). Results were expressed in EA units and were considered positive when more than 150 EA were detected.

Outcome measures

Regarding IgE test results, the following outcome measures (OM) were considered for each included cat:

1. OM1: Positive/negative for any food and/or environment allergen, excluding flea saliva
2. OM2: Positive/negative for any food allergen(s)
3. OM3: Positive/negative for any environment allergen(s)
4. OM4: Positive/negative for flea saliva allergen
5. OM5: For each cat, number of positive results for environment allergens
6. OM6: For each cat, number of positive results for food allergens
7. OM7: For each cat, number of positive results for both food and environment allergens
8. OM8: For each cat, sum of the 5 highest optic density (OD) values

Outcome measures OM5 to OM7 were used to take into account the possible poly-sensitizations in true hypersensitive cats, namely that these cats were sensitized for multiple allergens while non-hypersensitive cats might have been sensitized for fewer allergens. Additionally, OM8 was used to assess whether or not hypersensitive cats have higher OD values that asymptomatic animals.

Outcome measures OM7 and OM8 were also used to assess the influence of clinical and environmental factors on the overall level of allergen-specific IgE.

Tested hypotheses

Firstly, we wanted to determine whether or not some clinical features and environmental factors, namely age, gender, flea, and worm treatment, urban versus rural environment or indoor versus outdoor status influence the overall allergen specific IgE levels in pruritic cats. For this purpose, the correlation between OM7 and OM8 and age of individual cats was studied. For other potential risk factors the means of OM7 and OM8 of individual cats were compared between groups, namely male versus female, rural versus urban, etc.

Secondly, we wanted to test the hypothesis that the results of IgE testing may be helpful to predicting the final diagnosis. For this purpose, OM2 (yes/no) and OM6 (mean) were compared for Food HD cats (the control group in this case being formed by all other cats).

As well, OM3 (yes/no) and OM5 (mean) were compared for the group Non-Flea/Non-Food HD and a control group being formed by all other cats. For the last two analyzed, UHD cats were not taken into account.

Subsequently, OM4 (yes/no) was compared for Flea HD versus all other cats. Additionally, we compared OM7 and OM8 for all allergens together in cats from the hypersensitivity group (Non-Flea/Non-Food HD, Food HD and UHD) and from the non-hypersensitivity group. This was to examine if an allergen-specific IgE test could predict that one specific cat suffers a hypersensitivity disorder.

Statistical analyses

Means, proportions and correlations were analyzed using the Mann-Whitney test for non-parametric data, Fischer exact test, and Spearmann-rank correlation test, respectively. All analyses were carried out using Graphpad Instat software (GraphPad, San Diego, California, USA).

Results

Sera (n = 211) from pruritic cats were collected. Thirty-two cats with ambigious diagnoses or more than one diagnoses were excluded from the study. A total of 179 pruritic cats were divided into groups as shown in Table I. Additionally, sera of 20 healthy cats were analyzed.

Risks factors and allergen-specific IgE levels

For this part of the analysis, all cats (n = 199), irrespective of their health status, were taken into account.

Deworming status and allergen-specific IgE levels

When mean OM8 (sum of the 5 highest OD values) was compared for both dewormed [n = 87, mean: 5823 EA Units, standard deviation *(s)* = 5998] and non-dewormed cats (n = 112, mean: 3541 EA Units; s = 4341), the difference was statistically significant (P = 0.01; Mann-Whitney).

Also, when OM7 (number of positive results for both food and environment allergens) was compared for dewormed and non-dewormed cats, the difference was again statistically significant (means: 12.8/6.3; s = 13.4/9.7; P = 0.007; Mann-Whitney). This showed that non-dewormed cats are more prone to developing allergen-specific IgE.

Flea control and allergen-specific IgE levels

When mean OM8 from adequately (n = 96) and inadequately flea-controlled (n = 103) cats (before inclusion in the study) were compared, a significant difference was found (P = 0.03; means: 3564/4662; s = 2487/4001).

When mean OM7s from adequately and inadequately flea-controlled cats were compared, a significant difference was found (P = 0.05; means: 6.58/8.62; SD:6.01/8.2): these results suggested that inadequately flea-controlled cats have more positive results (OM7) and higher OM8.

Also, 29 cats out of 69 with not adequate flea control were positive for flea allergens, while only 26 out of 72 adequately flea-controlled cats were positive. This difference (Fischer's exact test) was statistically significant (P = 0.04).

Age and allergen-specific IgE levels

Correlation between age and the number of positive results OM7 was studied first. This correlation was 0.34 and considered very significant: $P < 0.0001$. Then correlation between age and OM8 was studied and we found a positive correlation (0.43) with a very significant P-value (< 0.0001).

These results suggest that cats are sensitized throughout life and that older cats more often test positive for allergen-specific IgE.

Gender and allergen-specific IgE levels

As far as OM7 and OM8 are concerned, differences between males (n = 95) and females (n = 104) were not significant.

Indoor versus outdoor status and allergen-specific IgE levels

When OM7 [mean: 4.3/9.1; standard deviation *(s)*: 8.4/11.9] and OM8 (mean: 3059/4813; s: 4222/5100) were compared between strictly indoor (n = 77) and outdoor or indoor/outdoor cats (n = 102),

Table I. Groups of included cats

Group	Number of cats
Non-flea/Non-food-induced hypersensitivity dermatitis[a] (Non-Flea/Non-Food HD)[b]	60
Food-induced hypersensitivity dermatitis (Food HD)[c]	15
Undetermined hypersensitivity dermatitis (UHD)[d]	70
Flea bite hypersensitivity dermatitis (Flea HD)[e]	16
Non-allergic pruritic cats (OD)[f]	18
Healthy controls	20
Total (all included cats)	199

[a] Skin inflammation caused by hypersensitivity (allergic) reaction.
[b] Cats with HD, suspected to be against environmental allergens.
[c] Cats with HD caused by food allergens.
[d] Cats with HD, suspected to be against environmental and/or food allergens.
[e] Cats with HD caused by flea saliva allergens.
[f] Cats with pruritus due to non-allergic cause.

difference was very significant ($P = 0.01$ and 0.01, respectively) in both cases. As well, OM1 was compared between indoor and outdoor cats. Outdoor cats had more positive results for food or/and environmental allergen-specific IgE (71 positive, 17 negative) compared with indoor cats (37 positive and 31 negative) with $P = 0.0008$ (Fischer's exact test).

Rural versus urban environment and allergen specific IgE levels

Cats were considered as living in a rural environment ($n = 76$) or in an urban one ($n = 129$). OM1, OM7, and OM8 were compared for both groups, but differences were not statistically significant.

Allergen-specific IgE and allergy diagnosis

Flea allergen-specific IgE testing for the diagnosis of Flea HD (flea bite hypersensitivity dermatitis)

Cats with Flea HD where compared to other allergic and non-allergic cats (healthy controls and pruritic cats with other diseases). For this analysis, results of the flea allergen-specific IgE test was interpereted as positive > 150 EA or negative < 150 EA (OM4). All comparisons demonstrated significant difference between Flea HD cats and other cats with a P-value ranging from 0.005 (comparison with non-allergic cats) to 0.0002 (all other cats or all other allergic cats).

When Flea HD cats were compared to all other cats, sensitivity and specificity of the serology test were 87% and 74%, respectively. In fact 14 out of 16 Flea HD cats were positive for flea allergens, while only 47 out of 183 Non-Flea HD cats were also deemed positive.

Allergen specific IgE testing for the diagnosis of Non-Flea/Non-Food HD

Non-Flea/Non-Food HD cats were compared to all other cats after UHD cats were excluded: differences were not significant (Table II). OM5 was additionally compared for Non-Flea/Non-Food HD alone and all other cats. Means between groups, however, were not statistically significant.

Table II. Comparison of groups in order to assess the reliability of allergen-specific IgE testing for the diagnosis of Non-Flea/Non-Food HD

Compared groups	OM3[a]-positive cats	OM3-negative cats
Cats with Non-Flea/Non-Food HD	30	30
All other cats (after UHD excluded)	45	24

[a] Environmental allergens.

These results indicate that allergen-specific IgE test cannot be used for the diagnosis of Non-Flea/Non-Food HD.

Allergen-specific IgE test for the diagnosis of food hypersensitivity

Food HD cats alone were compared to all other cats, after UHD cats exclusion and differences were not significant (Table III).

OM6 means of Food HD cats and Food HD + UDH were compared to other cats but differences again were not significant.

The results collectively suggest that allergen-specific IgE test is not an adequate test for the diagnosis of food hypersensitivity in cats.

Allergen-specific IgE test for the determination of a hypersensitivity disorder

In order to assess whether or not IgE tests can be used to determine a hypersensitivity status, 2 additional parameters were compared between allergic cats (Non-Flea/Non-Food HD, UHD, and Flea HD) and other cats: the total number of positive results on IgE test (OM7) and the sum of the 5 highest optic density values (OM8).

As far as the number of positive value is considered, means/SD were respectively: 7.26/11.2 and 7.7/9.8. Also mean/SD for allergy score were 4032/4861 and 3893/4698. Differences were not statistically significant.

Discussion

Feline skin hypersensitivity disorders are regarded as common and commercially available tests for the *in vitro* measurement of allergen-specific IgE are numerous. However, it is still not clear how useful these tests are, as the involvement of type I hypersensitivity and pathogenic role of IgE in all these disorders are not fully understood. Additionally, the level of allergen-specific IgE may be influenced not only by hypersensitivities to allergens, causing skin disorders — flea, food, and environmental allergens, but by endoparasitic infestation, and may possibly be influenced by some other factors, such as age, gender, and style of life. The last point was the first hypothesis we wanted to test in this study. Gilbert and Halliwell (15) showed, for example, that cats experimentally infected with *Toxacara canis* had enhanced food allergen-specific IgE responses. In our study absence of deworming is clearly associated with increased levels of IgE to non-worm allergens in cats. This possibly means that presence of intestinal parasites not only provokes parasitic IgE formation, as was already known, but also IgE to other allergens. As mentioned, positive correlation between infestation with *Toxocara canis* and *T. cati* and sensitization to orally administered antigens has already been proven (15). Our findings indicate that this correlation may also exist between endoparasitic

Table III. Comparison of groups in order to assess the reliability of allergen-specific IgE testing for the diagnosis of food hypersensitivity

Compared groups	OM2[a]-positive cats	OM2-negative cats
Food HD	5	10
All other cats (after UHD exclusion)	46	68

[a] Food allergens.

infestation and development of IgE to environmental allergens. As endoparasitic infestation has been shown to diminish the clinical signs of hypersensitivity in humans and dogs (16,17), it would have been interesting to compare clinical signs in dewormed and non-dewormed cats. This, however, was beyond the scope of this study and fecal examination was not carried out.

It is believed that type I hypersensitivity and IgE formation play central role in feline flea hypersensitivity dermatitis. Furthermore, a fairly good correlation between clinical disease and flea-specific IgE in serum has already been shown (18).

Our findings confirm those from previous studies and indicate that the IgE test can be considered as a reliable test for the diagnosis of flea bite hypersensitivity in cats. Interestingly, however, flea control status in our study did influence the IgE production, showing that presence of fleas might induce formation of IgE also to unrelated allergens, such as food and environmental allergens. These findings mirror those related to deworming. According to the findings herein, age influences levels of allergen-specific IgE in cats. The older the cat the more positive reactions and the higher IgE titers to food and environmental allergens. This age-related sensitization should probably be taken into account when IgE serology is interpreted. These findings may complicate the choice of allergens for desensitization in older cats, as some sensitization may not be related to the disease itself. There were no differences between males and females considering IgE levels, suggesting that gender does not influence levels of allergen-specific IgE expression. Although it is difficult to make a conclusion as most of the privately owned cats are spayed or neutered and are not under the same hormonal influence as an intact animal.

Way of life, namely indoor/outdoor status, clearly influenced allergen-specific IgE expression in our study. Outdoor cats had more positive reactions and higher allergen-specific IgE expression to food and environmental allergens than cats living strictly indoors. That probably reflects the ongoing challenge of outdoor cats with numerous environmental allergens, ecto-, and endoparasites.

There were no statistically significant differences in allergen-specific IgE expression between cats living in urban and rural environments. This could reflect the fact that cat's lifestyle is basically the same independent of where it lives, and the relative subjectivity of these terms (urban versus rural), considering contemporary urbanization.

It is very likely that some of the factors influencing allergen-specific IgE expression are linked together, which could be demonstrated using a multivariate analysis approach. The goal of our study, however, was to show that several factors should be taken into account when allergen-specific IgE test results are interpreted and to assess correlations among these factors.

In this study, half of the cats with a clinical diagnosis of Non-Flea/Non-Food HD were negative in IgE testing against environmental allergens and, vice versa, a large number of individuals not belonging to this group were positive for environmental allergens. This makes the IgE test an unreliable test for diagnosing of Non-Flea/Non-Food HD in cats. However, these data do not address the benefit of IgE testing for the selection of allergens for allergen-specific immunotherapy. This question was beyond the scope of this study and has already been demonstrated (2,9,10).

It could also been helpful to compare the results of an allergen-specific IgE test with an intradermal test. The latter has been shown to be more sensitive but less specific than the former in one experimental study (11). This has not been included herein because intradermal tests are known to be difficult to interpret and may be associated with huge interobserver variability (2). It would also have been interesting to evaluate these cats for allergen specific IgG because of their potential role in the development of some hypersensitivity reactions (8). This, however, was beyond the scope of this study because the main goal was to evaluate tests that are currently commercially available.

Little is known about mechanisms that induce pruritic dermatitis in cats following ingestion of food but considering that food hypersensitivity in cats is not always IgE driven (19) and that the reaction is not necessarily immunological, it seems logical not to expect an IgE test to be useful for diagnosing adverse food reactions. Poor correlation between serum food-specific IgE and adverse food reaction and also high percent of healthy control cats having food IgE has been reported (20). Although the number of food allergic cats in our study was quite limited, results confirm previous findings and indicate that IgE serology is an unreliable test for the diagnosis of food hypersensitivity in cats.

In conclusion, measuring allergen-specific IgE in cats as a part of allergic diagnostic work-up does not have significant value, except for assessing a role for flea allergens in a pruritic cat. It is clear, that production of IgE is influenced by different factors, such as age, flea control, and deworming status, way of life and probably by others, which are not known at the moment. This information should be taken in account when one considers evaluating the level of allergen-specific IgE in a feline patient. It should be stressed here that the only way to confirm the diagnosis of HD in cats is to rule out similar diseases and successfully treat these individuals. These results, however, do not prove that using this testing for the selection of allergens for desensitization of allergic cats is not adequate.

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Appendix I

1. Environmental allergens

1.1 Tree pollens

- 1.1.1 Birch *(Betula populifolia)*
- 1.1.2 Alder *(Alnus)*
- 1.1.3 Oak *(Quercus)*
- 1.1.4 Cypress *(Taxodium distichum)*
- 1.1.5 Hazel *(Corylus avellana)*
- 1.1.6 Elm *(Ulmus campestris)*
- 1.1.7 Beech *(Fagus sylvatica)*
- 1.1.8 Poplar *(Populus)*
- 1.1.9 Maple *(Acer pseudoplatanus)*
- 1.1.10 Willow *(Salix caprea)*
- 1.1.11 Olive *(Olea europaea)*
- 1.1.12 Red cedar *(Juniperus virginiana)*

1.2 Grass and weed pollens

- 1.2.1 Grass mix (Orchard grass, *Dactylis glomerata;* Medow fescue, *Festuca pratensis;* Perennial ryegrass, *Lolium perenne;* Timothy grass, *Phleum pratense;* Kentucky bluegrass, *Poa pratensis;* Velvet grass, *Holcus lanatus*)
- 1.2.2 Redtop *(Agrostis alba)*
- 1.2.3 Bermuda grass *(Cynodon dactylon)*
- 1.2.4 Johnson grass *(Sorghum halepense)*
- 1.2.5 Yellow Dock *(Rumex crispus)*
- 1.2.6 English plantain *(Plantago lanceolata)*
- 1.2.7 Mugwort *(Artemisia vulgaris)*
- 1.2.8 Lamb's Quarter *(Chenopodium album)*
- 1.2.9 Nettle *(Urtica dioica)*
- 1.2.10 Ragweed *(Ambrosia)*
- 1.2.11 Wall pellitory *(Parietaria officinalis)*
- 1.2.12 Russian Thistle *(Salsola kali)*

1.3 Molds

- 1.3.1 *Alternaria alternata*
- 1.3.2 *Cladosporium herbarum*
- 1.3.3 *Aspergillus fumigatus*
- 1.3.4 *Penicillium notatum*

1.4 Mites

- 1.4.1 *Dermatophagoides farinae*
- 1.4.2 *Dermatophagoides pteronyssinus*
- 1.4.3 *Tyrophagus putrescentiae*
- 1.4.4 *Lepidoglyphus destructor*
- 1.4.5 *Acarus siro*

1.5 Various

- 1.5.1 Flea saliva
- 1.5.2 Cat epithelium
- 1.5.3 Cockroach *(Blattella germanica)*

2. Food allergens

- 2.1 Beef
- 2.2 Chicken
- 2.3 Pork
- 2.4 Fish mix (tuna, cod, halibut)
- 2.5 Egg
- 2.6 Milk
- 2.7 Rice
- 2.8 Wheat
- 2.9 Corn
- 2.10 Soybean
- 2.11 White potato
- 2.12 Lamb

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